NT-proBNP in Fontan patients: heart failure or circulatory failure

Editorial

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The Fontan/Kreutzer operation was invented in 1971 as a palliative procedure to treat chronic cyanosis in patients with functionally univentricular cardiac defects, in whom biventricular repair was impossible. This operation changes circulatory characteristics so fundamentally, that it is warranted to call it the “Fontan Circulation”, that cannot by any means be regarded to function as a normal circulation. According to the existing guidelines for anatomically normal (biventricular) hearts, Fontan patients can be considered to be in ‘Heart Failure’ (HF) from the moment the operative sequence is completed. On top of that, due to the un-physiological characteristics of this circulation, these patients have proven to be prone to gradually increasing failure, which in itself differs fundamentally from the regular HF, and may result ultimately in transplantation or premature death. All the unique aspects of the Fontan Circulation have to be kept in mind when considering its failure and the value of potential biomarkers, which is the subject of the article of Heck et al in the current issue of the *European Journal of Heart Failure*.

Since its invention independently by both Francis Fontan and Guillermo Kreutzer in 1971 in France and Argentina, their operation has been performed in patients with a functionally single ventricle where, in the absence of a pulmonary (right) ventricle –at least of adequate size, the systemic venous return is surgically connected directly to the pulmonary circulation. This circulation does not exist naturally so can rightfully be called iatrogenic. The resulting characteristics of this iatrogenic circulation are a lower than normal cardiac index, limited capacity for the low output to rise during exercise and a central venous pressure which is chronically elevated because systemic venous return is subjected to pulmonary vascular resistance (PVR) without the aid of a ventricular pump. The preload is low because these single ventricles are usually anatomically larger than a normal ventricle. The ventricular afterload is increased by »10% because both pulmonary and systemic circulations are connected directly in series, while the PVR is »10% of systemic resistance.

The Fontan operation has proven to be palliative as these patients still face devastating complications developing gradually long after Fontan completion. A Fontan Circulation can fail on several weak points apparently inherent in its design. Firstly, the chronically increased central venous pressure due to the un-physiological circulation causes systemic venous congestion and it sequel such as lower body edema, ascites, protein losing enteropathy, hepatic cirrhosis and hepatic malignancy. Moreover, due to anatomical right atrial dilatation and previous surgical scarring, atrial arrhythmias occur frequently, which in turn has a detrimental effect on ventricular filling. Secondly, Fontan patients are often faced with the consequences of decreasing myocardial function (either systolic or diastolic) over time, the mechanism of which still needs to be elucidated. The third mechanism is the gradual increase of the PVR, caused possibly by pulmonary vascular remodeling most likely due to the non-pulsatile character of the pulmonary flow and to chronic pulmonary (micro-)emboli. Finally, a dysregulation of the autonomic nervous system and the neurohumeral axis may play a role in failure of this unique circulation on the long term. Of all Fontan patients, about half is alive 25-30 years after initiation of this aberrant circulation. Therefore, this Fontan failure asks for more elucidation, and the identification of potential biomarkers for failure will be of great value for the management of these patients.
Heck et al investigated in this issue the value of NT-proBNP in a cohort of 124 patients, 13.8 ± 7.3 years after Fontan surgery. NT-proBNP is a well recognized biomarker for HF as this hormone is released from cardiac muscle, particularly the ventricles, but also the atria. According to guidelines existing for the normal circulation, from the moment the operative sequence leading to the Fontan circulation is completed, the Fontan patient shows resemblance to HF patients, in terms of symptoms (e.g. breathlessness and fatigue on exercise) and signs (e.g. elevated jugular venous pressure) that both result from an “abnormality in cardiac structure or function”. Is it possible that HF biomarkers like NT-proBNP could play a similar role to the Fontan patients as to the HF patient we know from daily our cardiological practice? If Fontan failure would be characterized by either ventricular or atrial volume overload causing wall stretch, NT-proBNP could be a tell-tale for failure.

In the study by Heck et al, the type of Fontan surgery appeared to be a crucial factor in the interpretation of the NT-proBNP level. Therefore, it is of value to sketch some of the surgical modifications that were developed in the 42 years since its concept. This evolution can be typified by varying amounts of atrial tissue incorporated in the connection between the inferior caval vein (IVC) and the pulmonary artery. The original Fontan circuit existed of an atrio-pulmonary connection connecting the right atrial auricle to the pulmonary arteries directly. This operation ultimately results invariably in a hugely dilated right atrium, functioning in itself as a substantial resistance and risk for trombo-emboli. The so-called “lateral tunnel” technique was developed by Marc de Leval in order to replace the huge atrium with a low resistance tubular pathway between the IVC and the pulmonary artery, in which the tube was built from prosthetic material and a portion of the lateral atrial wall. Finally, the extracardiac conduit was conceived in the Mayo Clinics, consisting purely of a prosthetic tube connecting the IVC with the pulmonary arteries. Both the lateral tunnel and the extracardiac tube are operative techniques currently en vogue. Heck et al stated that these differences in the incorporation of myocardium in the Fontan circuit would influence the diagnostic value of the biomarker originating from the cardiac muscle. They showed that the older Fontan modifications that involve more atrial tissue in the systemic venous pathway, show higher NT-proBNP, independent of the patient’s ventricular function.

When considering these results, one has to keep in mind that the Fontan patients have limited survival and the patients who are operated with the older techniques are in a different phase of their Fontan Circulation than are the patients operated with the newer techniques. This is also reflected by a difference in ventricular function between these different groups. So not only the incorporation of atrial wall into the Fontan circuit, but also the interval since the Fontan operation and thereby the degree of attrition and associated decline in ventricular function, may have influenced the NT-proBNP levels in the patients with atrio-pulmonary or atrioventricular connections.

Besides, the aortopulmonary flow may influence the NT-proBNP as a biomarker in Fontan Failure. Collateral flow can be present in Fontan patients and can have accumulated to substantial proportions, particularly in the cyanotic phase of these patients. These possibly
vast amounts of collateral flow could cause an additional volume load to the single ventricle supplanting the initial underload. Thereby the pressure on the ventricular wall increases and more NT-proBNP could be disseminated. The amount of this collateral flow is still cumbersome to quantify and is thus a confounder of unknown magnitude.

As we have mentioned above, the Fontan Circulation has several modes of failure and it would be extremely valuable to identify biomarkers which are associated with a specific mode of failure. NT-proBNP, reflecting a high volume load and myocardial wall stress, may be disseminated in every mode of failure because of the serial direct coupling of systemic and pulmonary circulation. Every increase in PVR will lead to more congestion in the body and therefore in increasing ventricular afterload and myocardial stress. In the future, biomarkers which discriminate between the different modes of failure of the Fontan Circulation could be a focus point for further research. However, identifying and quantifying the failure of the Fontan Circulation is not a one-dimensional process, but asks for an all-encompassing view with all the different aspects of the Fontan Circulation kept in mind.
References


In the Fontan circulation, NT-proBNP serum level reflects circulatory performance more than intrinsic cardiac function.
Abstract

Background: In Fontan patients, the peptide N-Terminal pro Brain Natriuretic Peptide (NT-proBNP) is often increased, but its value in the assessment of cardiac function and circulatory performance is unclear. The purpose of this study in Fontan patients is to investigate whether NT-proBNP represents cardiac function or other key variables of the Fontan circulation and is related to functional status.

Methods and results: Ninety-five consecutive Fontan patients ≥ 10 years old who attended the pediatric or cardiologic outpatient clinic in 2012-2013, were included. Time since the Fontan operation was 16 ± 9 years. Median NT-proBNP was 114 (IQR 61-264) ng/l, and was higher than age-dependent normal values in 42% of the patients. Gender- and age adjusted logNT-proBNP levels were not related to functional status, defined as NYHA functional class, peak exercise capacity and cardiac index, nor to cardiac function, assessed by cardiac magnetic resonance (ejection fraction) and echocardiography (ventricular function, E’, E/E’ ratio, E/A ratio). However, peptide levels significantly correlated with indexed ventricular mass (p=0.001), inferior caval vein diameter (p < 0.001) and gamma-glutamyl transferase (p=0.011, adjusted Rsquare=0.636).

Conclusion: In patients with a Fontan circulation, NT-proBNP is increased in 42% of the patients, and progressively increases with higher patients’ age or longer time since Fontan operation. NT-proBNP relates to the circulatory performance of the Fontan circulation, and might signal early attrition of this circulation. However, it shows limited association with intrinsic cardiac function. Therefore, NT-pro-BNP, one of the most important biomarkers of biventricular heart failure, requires a different interpretation in patients with univentricular physiology.
Introduction

Patients born with a univentricular heart usually require advanced cardiac surgery, known as the Fontan operation. Although the Fontan operation successfully treats cyanosis and ventricular volume overload, it is a palliative procedure. Eventually, attrition of the circulation could lead to a constellation of serious symptoms and findings known as "Fontan Failure." Fontan Failure can include ventricular dysfunction, protein losing enteropathy, hepatic failure and a gradual increase in pulmonary vascular resistance. Timely recognition of Fontan failure may be of help to initiate early treatment and improve survival.

A valuable aid in the recognition of a failing Fontan circulation might be N-Terminal pro Brain Natriuretic Peptide (NT-proBNP). NT-proBNP is a peptide that is released from the cardiac tissue in response to wall stress due to volume- or pressure-overload. In patients with biventricular circulation, NT-proBNP is a well-recognized biomarker, elevated in patients with heart failure with reduced and reserved ejection fraction and has become crucial for decision making in current clinical practice. However, in the univentricular physiology, the role of NT-proBNP in the assessment of cardiac function and circulatory failure is far from unraveled. Due to the unique characteristics of the Fontan circulation, including the serial coupling of pulmonary and systemic circulation, different surgical techniques, and abnormal ventricular loading conditions, the value of NT-proBNP in biventricular hearts may not be extrapolated without further consideration.

The purpose of this study in Fontan patients is to investigate whether NT-proBNP represents cardiac function or other key variables of the Fontan circulation and is related to functional status.

Methods

Patient population

Consecutive patients ≥ 10 years old with a Fontan circulation, attending the outpatient clinic of the University Medical Center Groningen between January 2012 and October 2013, were included in the current cross-sectional study. During these visits, each patient underwent a standardized set of tests, including transthoracic echocardiography, cardiopulmonary exercise testing, cardiac magnetic resonance examination, and laboratory measurements. This study complies with the declaration of Helsinki. The institutional ethics committee approved the conduct of this investigation. Informed consent was obtained from all study participants and/or their parents.

Clinical data

Patient characteristics. Data on demographics and clinical variables were extracted from medical records. These data included age, sex, body mass index (BMI), cardiac anatomy, type of initial
Fontan operation and follow-up since the Fontan operation. Body surface area (BSA) was calculated with Haycock’s formula\(^7\).

**Functional status.** Functional status was defined as the New York Heart Association (NYHA) functional class and maximal oxygen uptake during exercise (peak VO\(_2\)). The NYHA functional class was assessed by two experienced physicians. The peak VO\(_2\) was measured during cardiopulmonary exercise testing using a stationary cycle ergometer in children < 18 years old, and a treadmill in adults. The maximum oxygen uptake was calculated as the mean of the two highest VO\(_2\) measurements during exercise, and indexed by body weight. The peak VO\(_2\) index is presented as absolute value and as percentage of predicted compared to normal values\(^8,9\).

**Laboratory measurements** Venous blood samples were drawn from all patients during routine follow-up. For NT-proBNP measurements, the specimens were collected in 4.5 ml lithium-heparin tubes, centrifuged and directly determined by immunosay using the Roche Modular E (Roche Diagnostics, Mannheim Germany). For the present study, we investigated two cut-off values for elevated NT-proBNP levels: the 97.5\(^{th}\) percentile of age-adjusted reference values\(^10,11\), and levels > 125 ng/l (according to the optimal cut-off point for non-acute presentation of biventricular heart failure)\(^6\). Additionally, potential confounders in NT-proBNP measurements, including anemia (hemoglobin) and renal function (creatinin, estimated glomerular filtration rate (eGFR)), and hepatic measurements associated with systemic venous congestion (gamma-glutamyl transferase (γ-GT)) were measured.

**Echocardiography.** Transthoracic echocardiography was performed using a commercially available General Electric ultrasound machine with a 3.5 MHz probe. A standardized protocol was used, which included parasternal, apical, subcostal and suprasternal views. Analysis of echocardiographic images was performed offline by an experienced sonographer who was blinded for all other study information. Systolic function was estimated (eyeballing) and classified as no, mild, moderate or severe dysfunction. For the assessment of diastolic function, the peak early (E) and late inflow velocities (A) across the dominant atrioventricular valve were measured using a pulsed Doppler sample on the apical chamber view. Pulsed wave Tissue Doppler Imaging was used to assess the peak early (E’) and late (A’) diastolic velocities of the atrioventricular annulus of the non-septal (free) wall. The E/E’ ratio and E/A ratio were calculated.

**Cardiac Magnetic Resonance examination (CMR).** All studies were performed on a 1.5 Tesla system (Siemens, Magnetom Avanto, Erlangen, Germany). The CMR protocol included a stack of short-axis slices from the base of the heart to the apex of the heart using cine-steady-state free precession (SSFP) with end- expiratory breath holding. The following scan parameters were used: slice thickness 6 mm; slice gap 4 mm; TR 2.7-3.4 ms; TE 1.1-1.7 ms; flip angle 80-90; matrix 171-192; voxel size 1.25x1.25x8.0 mm and 1.7x1.7x6.0 mm. No sedation was applied. Commercially available software was used for the imaging analysis (Qmass, version 7.6. Medis Medical Imaging Leiden, the Netherlands). End-systolic and end-diastolic phase were visually selected, using the largest and smallest systemic ventricular cavity on the longitudinal and short axis views. The contours of the systemic and hypoplastic ventricle
were manually drawn on epicardial and endocardial borders from the most apical to the most basal short axis slice. The end-systolic and end-diastolic blood volumes were calculated from the endocardial contours; both the volumes of the systemic and hypoplastic ventricle were included except for patients with pulmonary atresia and intact ventricular septum. Ventricular mass, including trabecular and papillary tissue, was calculated by using MassK semi-automatic threshold-based segmentation software\textsuperscript{12,13}. The ejection fraction (EF) and cardiac index (CI) were calculated from end-diastolic and end-systolic blood volume, heart rate and BSA.

In addition, measurements of the inferior caval vein (VCI) circumference were performed on the magnetic resonance angiography source image data in order to estimate the degree of systemic venous congestion. Double-oblique short axis of the VCI were determined in the 3D setting (Aquarius iNtuition software, TeraRecon, San Mateo, California, USA) with the long axis oriented from the mid-vessel on the sagittal plane and the short axis oriented perpendicular to the vessel wall on the oblique plane. The circumference of the vessel was measured between either the right atrium or extracardiac conduit anastomosis and the hepatic vein influx.

**Statistical analyses**

All statistical analyses were performed using SPSS for Windows (version 22, SPSS inc, Chicago, Illinois, USA). Continuous data were reported as mean ± standard deviations (SD) or median (interquartile range, IQR) and categorical data as number of patients (percentage of total number of patients). The primary outcome parameter was NT-proBNP. The NT-proBNP levels had an exponential distribution and were therefore transformed into logarithmic values. The covariates included 1) patient characteristics (e.g. type of and time since initial Fontan operation), 2) functional outcome (NYHA functional class, peak VO\textsubscript{2} index, CI), 3) cardiac parameters (CMR derived EF, end-diastolic volume (EDV) and ventricular mass, and echocardiographic eyeballing ventricular function, E', E/E' ratio, E/A ratio), 4) and variables associated with systemic venous congestion (VCI diameter, γ-GT levels). The type of initial Fontan operation was categorized by the amount of atrial tissue incorporated in the systemic-to-pulmonary tunnel, i.e. a total autologous tunnel (atriopulmonary connection, Björk modification, right auricular-VCI conduit\textsuperscript{14}), partial autologous tunnel (TCPC lateral tunnel) and no atrial tissue in the tunnel (TCPC extracardiac conduit and Kawashima).

The correlations between logNT-proBNP levels and the covariates were tested one by one using both univariable linear regression (model 1) and multivariable linear regression analyses in which gender and age were forced in the model to provide gender- and age adjusted correlations (model 2). Interaction was tested between covariates and gender as well as age. Finally, covariates showing a correlation with gender- and age adjusted logNT-proBNP levels with a significance of $p < 0.10$, were tested in a multivariable model using a backwards approach. Additionally, the multivariable model was tested with adjustment for other parameters known to influence NT-proBNP levels (kidney function and BMI). A probability value $< 0.05$ was considered significant.
Results

Patient characteristics

Ninety-five patients were included in the current study; 39 children (< 18 years old) and 56 adults. Patient characteristics are displayed in table 1. The patients underwent the Fontan operation between 1975 and 2010, at a median age of 4.5 (IQR 3.4-6.7) years. Time since the Fontan operation was 16 ± 9 years, with a minimum of 2 years and a maximum of 37 years. Sixty-two patients (65%) underwent cardiac magnetic resonance examination (CMR). The other patients did not participate in the CMR analyses because of an implanted pacemaker or pacemaker leads in 23 patients (24%), claustrophobia in 2 patients (2%), and patient refusal in 7 patients (7%). The median time between laboratory measurements and CMR was within 24 hours (IQR 0-26 days).

Clinical variables

Functional status
Thirty-six patients (38%) were in NYHA class I, forty-eight patients (51%) were in NYHA class II, and eleven patients (12%) were in NYHA class III. Peak VO\textsubscript{2}index was 25.2 ± 8.5 ml/min/kg (58 ± 14% of predicted). Mean cardiac index was 3.1 ± 0.9 ml/min/m\textsuperscript{2}.

Laboratory measurements
Median NT-proBNP was 114 (IQR 61-264) ng/l, with a minimum of 9 ng/l and a maximum of 1862 ng/l. Forty patients (42%) had a NT-proBNP level above the 97.5\textsuperscript{th} percentile of age-adjusted normal values and 42 patients (44%) had a NT-proBNP level higher than 125 ng/l. One patient (1%) showed signs of kidney dysfunction, with an increased creatinine level 162 µmol/l and a decreased eGFR of 30 ml/min/1.73m\textsuperscript{2}. Median γ-GT was 63 (IQR 38-108) U/l, and 59 patients (62%) had a γ-GT above the reference range.

Cardiac imaging
Mean CMR-derived ventricular ejection fraction was 56 ± 8%. All patients had an ejection fraction > 40%. Median indexed mass was 63 (IQR 54-73) gr/m\textsuperscript{2} and median indexed EDV was 74 (IQR 61-88) ml/m\textsuperscript{2}. VCI calculated diameter was 24 (IQR 21-31) mm. Using echocardiography, eyeballing estimation of the ventricular function revealed that 10 patients (11%) had a moderate/severe systolic dysfunction. Diastolic Doppler velocities showed a mean E’ of 10.8 ± 3.4 cm/s, median E/A ratio of 1.4 (IQR 1.1-1.8) and median E/E’ ratio of 6.2 (IQR 5.2-8.2).

Correlation analyses

Correlation with patient characteristics
Figure 1 displays the correlation between NT-proBNP and age of the Fontan patients, in relation to the age-appropriate reference values. The number of patients with NT-proBNP above the age-appropriate reference values increased significantly with increasing age (r=0.509, p < 0.001). The univariable and gender- and age adjusted regression analyses for logNT-proBNP level are displayed in table 2.
Table 1. Patient characteristics (n=95)

<table>
<thead>
<tr>
<th>Male</th>
<th>47 (50%)</th>
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**Diagnosis**

| TA | 41 (43%) |
| DILV | 22 (23%) |
| AVSD / unbalanced VSD | 12 (13%) |
| PA with IVS | 10 (11%) |
| Heterogeneous anomalies | 6 (6%) |
| HLHS | 4 (4%) |
| Heterotaxy | 14 (15%) |

**Ventricular morphology**

| Left dominant | 78 (82%) |
| Right dominant | 17 (18%) |

**Type of Fontan procedure**

| TCPC right auricular-VCI conduit | 27 (29%) |
| TCPC lateral tunnel | 22 (23%) |
| TCPC extracardiac conduit | 22(23%) |
| Atriopulmonary connection | 15 (16%) |
| Kawashima | 5 (5%) |
| Björk modification | 4 (4%) |

**Age at Fontan procedure, years** 4.5 (3.4-6.7)

**Current age, years** 21.6 (14.4-27.1)

**BMI** 20.9 (18.1-23.5)

**Body surface area** 1.66 ± 0.31

**Hemoglobin, mmol/l** 9.4 (8.9-10.1)

**Creatinin, umol/l** 64 (54-76)

**Gamma-glutamyl transferase, U/l** 63 (38-108)

**NT-proBNP, ng/l** 114 (IQR 61-264)

(A)VSD=(atrial)ventricular septal defect; BMI=body mass index; DILV=double inlet left ventricle; HLHS=hypoplastic left heart syndrome; IVS=intact ventricular septum; NT-proBNP=N-terminal pro brain natriuretic peptide; PA=pulmonary atresia; TA=tricuspid atresia; TCPC=total cavopulmonary connection.
Figure 1. Relation between age and NT-proBNP levels

CI=confidence interval; NT-proBNP=N-terminal pro brain natriuretic peptide.

Figure 2. Relation between operative technique and NT-proBNP levels

*P-value<0.05. APC=atriopulmonary connection; EC=extracardiac conduit; LT=lateral tunnel; NT-proBNP=N-terminal pro brain natriuretic peptide; RA=right auricular-VCI tunnel; TCPC=total cavopulmonary connection.
Table 2. Regression analyses

<table>
<thead>
<tr>
<th>Model 1</th>
<th>Model 2</th>
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<tbody>
<tr>
<td>Regression coefficient (95%CI)</td>
<td>Regression coefficient (95%CI)</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td><strong>P-value</strong></td>
</tr>
<tr>
<td><strong>Patient characteristics</strong></td>
<td><strong>Functional status</strong></td>
</tr>
<tr>
<td>Female gender</td>
<td>0.66(1.04-0.29)</td>
</tr>
<tr>
<td>Right ventricular morphology</td>
<td>0.45(-0.06-0.96)</td>
</tr>
<tr>
<td>Atriopulmonary connection</td>
<td>1.14(0.68-1.60)</td>
</tr>
<tr>
<td>Years since Fontan procedure</td>
<td>0.06(0.04-0.08)</td>
</tr>
<tr>
<td>Current age</td>
<td>0.05(0.04-0.07)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.06(0.01-0.10)</td>
</tr>
<tr>
<td>Body surface area</td>
<td>0.63(0.00-1.27)</td>
</tr>
<tr>
<td>Creatinin</td>
<td>0.02(0.01-0.03)</td>
</tr>
<tr>
<td><strong>Cardiac parameters</strong></td>
<td><strong>Variables associated with systemic venous congestion</strong></td>
</tr>
<tr>
<td>E/A ratio</td>
<td>-0.48(-0.91- -0.05)</td>
</tr>
<tr>
<td>Ventricular mass index</td>
<td>Ns</td>
</tr>
<tr>
<td>VCI diameter</td>
<td>0.07(0.04-0.11)</td>
</tr>
<tr>
<td>γ-GT</td>
<td>0.00(0.00-0.01)</td>
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</tbody>
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Model 1: Univariate regression analyses, Model 2: Regression analyses adjusted for gender and age.

BMI=body mass index; CI=confidence interval; E/A ratio= ratio of early / atrial filling velocity; γ-GT=gamma glutamyl transferase; NT-proBNP=N-terminal pro brain natriuretic peptide; NYHA-FC=New York Heart Association functional class; VCI=inferior caval vein; VO2= oxygen uptake during exercise.
The median NT-proBNP levels was 148 (83-381) ng/l in the patients with a completely autologous conduit (atriopulmonary connection/Björk modification/right auricular-VCI conduit), and was significantly higher compared to a median NT-proBNP of 103 (55-290) ng/l in patients with a partial autologous conduit (TCPC lateral tunnel, p=0.011) and 85 (51-135) ng/l in patients with a non-autologous conduit (TCPC extracardiac conduit/Kawashima; p < 0.001, figure 2).

Figure 3a. Relation between indexed ventricular mass and NT-proBNP levels

![Figure 3a](image)

NT-proBNP=N-terminal pro brain natriuretic peptide.

Figure 3b. Relation between VCI diameter and NT-proBNP levels

![Figure 3b](image)

NT-proBNP=N-terminal pro brain natriuretic peptide; VCI=inferior caval vein.
However, the gender- and age adjusted analyses regression analyses for logNT-proBNP levels showed no difference between these groups. The following variables showed no relation with (gender- and age adjusted) NT-proBNP levels: diagnosis, heterotaxy, age at Fontan completion, and hemoglobin.

**Correlation with functional status.** NT-proBNP levels were 84 (49-139) ng/l in patients in NYHA functional class I, 120 (65-269) ng/l in patients in NYHA functional class II, and 312 (158-581) ng/l in patients in NYHA functional class III (p=0.003). Both peak VO$_2$ index and cardiac index correlated with NT-proBNP levels (r=-0.319, p=0.002 respectively r=-0.279, p=0.030). The correlations between NT-proBNP and functional status faded in gender- and age adjusted analyses. Peak VO$_2$ index as percentage of predicted showed no significant correlation with NT-proBNP levels.

**Correlation with cardiac parameters** In univariable analyses, logNT-proBNP levels correlated with E/A ratio (r=-0.281, p=0.028). However, in gender- and age adjusted analyses, both systolic and diastolic functional cardiac parameters did not show significant correlations with logNT-proBNP levels. Indexed ventricular mass significantly correlated with gender- and age adjusted logNT-proBNP levels (figure 3A r=0.349, p=0.002).

**Correlation with variables associated with systemic venous congestion.** VCI diameter was significantly correlated with gender- and age adjusted logNT-proBNP levels (figure 3B r=0.335, p=0.010). γ-GT levels showed a positive trend towards correlation with gender- and age adjusted logNT-proBNP levels (r=0.182, p=0.057). The multivariable model for gender- and age adjusted logNT-proBNP is displayed in table 3. Of all included variables (ventricular morphology, γ-GT, VCI diameter and indexed ventricular mass), the γ-GT, VCI diameter and indexed ventricular mass contributed significantly to the model (Rsquare=0.636). The addition of BMI and kidney function did not change the contribution of the above mentioned variables in the multivariable model.

**Table 3. Multivariable model for logNT-proBNP, gender- and age adjusted**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standardized regression coefficient (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indexed ventricular mass, gr/m$^2$</td>
<td>0.311</td>
<td>0.001</td>
</tr>
<tr>
<td>VCI diameter, mm</td>
<td>0.328</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>γ-GT</td>
<td>0.252</td>
<td>0.011</td>
</tr>
</tbody>
</table>

Adjusted Rsquare=0.636

CI=confidence interval; γ-GT=gamma glutamyl transferase; NT-proBNP=N-terminal pro brain natriuretic peptide; VCI=inferior caval vein.
Discussion

The current study showed that NT-proBNP is increased in 42% of the Fontan patients, and the number of patients with NT-proBNP above the age-appropriate reference values increased significantly with increasing age. The gender and age-adjusted peptide levels did not correlate with Fontan operative technique, functional status, and cardiac function in patients with a Fontan circulation. Nevertheless, they did correlate with ventricular morphology, indexed ventricular mass, VCI diameter and \( \gamma \)-GT levels.

The Fontan circulation is associated with unique characteristics that hamper the extrapolation of NT-proBNP interpretation from the biventricular heart failure to the Fontan circulation. NT-proBNP is released from the ventricular and, in lower concentrations, atrial myocardium in response to increased wall stress caused by volume or pressure overload\(^{15-17} \). Nowadays, this peptide is one of the pillars of cardiologic clinical practice\(^6 \), where it is acknowledged as an important aid in the assessment of biventricular patients with (suspected) congestive heart failure, to evaluate the effects of therapy and to predict survival\(^{18-20} \). Congestive heart failure in biventricular patients is characterized by systolic or diastolic ventricular dysfunction and an increased end-diastolic volume or pressure, causing ventricular wall stress and consequently increased levels of NT-proBNP\(^{21,22} \). In contrast, in Fontan patients, the serial coupling of both systemic and pulmonary circulation causes a chronically restricted ventricular preload, systemic venous congestion and increased ventricular afterload. The univentricular physiology is therefore associated with several unique characteristics that have to be kept in mind when evaluating NT-proBNP levels. Firstly, a single ventricle sustains both the systemic and pulmonary circulation. Due to the direct coupling of both circulations, failure of the Fontan circulation concerns failure of the whole circulation and does not signify left- nor right-sided congestive heart failure\(^{23,24} \). Secondly, the evolution of the Fontan operation from an atriopulmonary connection to the total cavopulmonary connection is characterized by various amounts of native atrial tissue incorporated in the conduit between the inferior caval vein and the pulmonary artery. Because this atrial tissue is subjected to higher pressures, it might increase NT-proBNP levels. Thirdly, the univentricular heart is exposed to unique loading conditions that affect the volume and pressure load of the single ventricle.

In the current study, we found around 40% of the patients with a Fontan circulation to have increased levels of NT-proBNP. This is in accordance with previous studies, which showed increased peptide levels in 20-60% of the Fontan patients\(^{25,26} \). Univariable analysis confirmed that the peptide levels are inversely related to the time since Fontan operation\(^{25,26} \). That correlation, however, faded after adjustment for gender and age. Since age also reflects the time since Fontan operation, and the attrition of the Fontan circulation develops gradually in time, the interpretation age-adjusted NT-proBNP is multi-dimensional. In the current study, with longer time since the Fontan operation, an increasing number of patients appeared to have NT-proBNP levels that where increasingly higher than the reference range. This suggests a gradual attrition of the circulation. Our results suggest that, in Fontan patients, NT-pro-BNP level is correlated with functional status, but that this is not independent from the time since Fontan operation.
Concerning the relation between surgical techniques, NT-pro-BNP level seems not primarily to be determined by the amount of remaining native atrial tissue, but to be more dependent of age or the time since Fontan operation. Indeed, patients with a TCPC using a native right auricular tunnel, that were operated relatively recently \(^{14}\) had comparable NT-proBNP levels to patients with extracardiac conduits, operated in the same era. Therefore, surgical technique was not included as a variable in the multivariable model.

In the Fontan circulation, systemic venous congestion does not signify increased ventricular preload to the systemic ventricle. On the contrary, the direct coupling of the systemic and pulmonary circulation results in a restricted ventricular preload due to the passive pulmonary blood flow\(^{27}\). Furthermore, to overcome the pulmonary vascular resistance, increased systemic venous congestion develops. Finally, the serial coupling and presumably an autonomic deregulation in order to maintain blood pressure in low cardiac output state, induce an increased ventricular afterload\(^{27-29}\). The current study demonstrated no correlations between NT-proBNP and cardiac function variables, in these unique ventricular loading conditions. Only a weak correlation with E/A ratio existed. Our results correspond with previous reports, which observe no or only weak relations between NT-proBNP levels and ventricular function\(^{30-34}\). Concerning systolic function, none of the patients in the current study had ventricular dysfunction (EF < 40\%), which might have limited the power of the analyses. Regarding diastolic function, a previously proposed concept, describing diastolic dysfunction in Fontan patients to be caused by a downwards spiral of ventricular disuse hypofunction due to the restricted ventricular preload\(^{35}\), might explain why we found a weak correlation with NT-proBNP levels. It is important to realize that the most frequently used non-invasive parameters of cardiac function are load-dependent and in the Fontan circulation, the loading conditions differ significantly from the normal circulation. This might contribute to the lack of correlation between NT-proBNP and these cardiac functional variables in Fontan patients.

With attrition of the Fontan circulation, the unique ventricular loading conditions may further deteriorate. Pulmonary vascular remodeling in patients with a longstanding Fontan operation has been recently identified\(^{36}\). Progressive pulmonary vascular remodeling might cause the pulmonary vascular resistance to rise\(^{37}\), thereby increasing systemic venous congestion and ventricular afterload. In the current study, we showed that NT-proBNP levels in the Fontan patients were significantly related to ventricular mass, VCI diameter and γ-GT. Increased ventricular mass might either be inherent to the univentricular heart, or a response to an increased ventricular afterload. Increased afterload may cause an increase in ventricular wall stress and, consequently, higher NT-proBNP levels in Fontan patients. This concept is endorsed by previous findings, including the significant relation between NT-proBNP levels and end-diastolic pressure in the systemic ventricle during cardiac catheterization\(^{33,38}\) and the correlation between ventricular mass and NT-proBNP in children with a Fontan circulation\(^{34,39}\). Both VCI diameter and γ-GT represent systemic venous congestion\(^{40,41}\). The correlation between these variables and NT-proBNP indicates that this peptide might be valuable in the recognition of Fontan failure by signaling a less-optimal circulatory performance of the Fontan circulation. Furthermore, increased systemic venous congestion is associated with hepatic and enteric associated morbidity and mortality\(^{42-44}\).
which might explain why NT-proBNP levels predicts worse outcome in the Fontan patients.25,26. Finally, we identified that increased levels of NT-proBNP were associated with right ventricular morphology. This might indicate that the right ventricle suffers more from the abnormal loading conditions associated with a systemic single ventricle, and might be associated with an earlier deterioration of the circulatory performance of the Fontan circulation.

For clinical practice, the results of the current study indicate that NT-proBNP levels relate to the circulatory performance of the Fontan circulation and might signal early attrition of this circulation. However, NT-proBNP seems of limited value to assess intrinsic cardiac function in Fontan patients. Since the patients’ age also reflects the time since Fontan operation and associated attrition of the circulation, the age-adjusted NT-proBNP levels have to be interpreted with care.

**Limitations**

The current study is a cross-sectional study. Changes in NT-proBNP levels over time in individual patients, which would be valuable to assess the underlying mechanism of changes in NT-proBNP and ventricular dimensions, could not be addressed due to this study design. Furthermore, in some cases, the multivariable regression analyses were limited to the patients who underwent CMR imaging (n=62). Previous studies have primarily focused on echocardiographic measurements of cardiac dimensions, and, despite this limited number of patients, we were able to identify significant relations. To our knowledge, the current study is the first to show the relation between peptide levels and variables associated with systemic venous congestion in the Fontan circulation. Finally, no catheterization data were available for this cohort of patients at the time of NT-proBNP measurement. We recommend future studies which include catheterization measurements to aim at investigating the relation between changes in catheterization derived hemodynamics and NT-proBNP levels.

**Conclusions**

In patients with a Fontan circulation, NT-proBNP is increased in almost half of the patients, and progressively increases with higher patients’ age or longer time since the Fontan operation. These NT-proBNP levels relate to the circulatory performance of the Fontan circulation, and might signal early attrition of this circulation. However, they show limited association with intrinsic cardiac function. Therefore NT-pro-BNP, one of the most important biomarkers of biventricular heart failure, requires a different interpretation in patients with univentricular physiology.
References


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